

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	
<b>CHEN et al.</b>	
Patent No.:	<b>7,595,179</b>
Issue Date:	<b>September 29, 2009</b>
For: <b>RECOMBINANT REVERSE TRANSCRIPTASES</b>	

Customer No.: 68163

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**REQUEST FOR CERTIFICATE OF CORRECTION UNDER  
35 U.S.C. 255 and 37 C.F.R. § 1.323**

Commissioner for Patents  
**ATTN: CERTIFICATE OF CORRECTIONS BRANCH**  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

Patentees request a Certificate of Correction to correct errors set forth in the attached form PTO/SB/44 (Rev. 04/05). Said errors are on the part of the Patent Office and the Patentee.

The error at column 5, line 62, is a typographical error on the part of Patentee. Basis for the requested correction is present at column 5, line 60, which line refers to an RT-PCR kit, thereby providing proper antecedent basis for "RT-PCR" buffer.

The error in SEQ ID NO:3 and the error in SEQ ID NO:5 at column 16, lines 15 and 22, respectively, are on the part of the Patent Office. Basis for the requested corrections to SEQ ID NO: 3 and SEQ ID NO: 5 at column 16, lines 15 and 22, respectively, is present in the specification as originally filed. For the convenience of the examiner, provided herewith is a copy of page 21 of the specification as originally filed containing the sequence of SEQ ID NO:3 and of SEQ ID NO:5 at lines 28 and 32, respectively. The error in SEQ ID NO:3 as printed in the patent is the omission of two "C's" at nucleotide 18. The error in SEQ ID NO:5 as printed in the patent is the addition of an extra "T" at nucleotide 34. SEQ ID NO:3 and SEQ ID NO:5 are correct in the Sequence Listing of the Patent.

The error in Claim 22 at column 38, line 8, is an informal error in printing by the Patent Office where a space is missing between the words "the" and "reaction." Basis for the

requested correction to Claim 22 is found in the attached copy of the Response to Final Office Action mailed January 8, 2009 at then numbered Claim 154 (issued as Claim 22).

The error in Claim 41 at column 38, line 52, is a typographical error on the part of Patentee. Basis for the requested correction is present in Claim 41, column 38, line 49, which line refers to an RT-PCR kit, thereby providing proper antecedent basis for "RT-PCR" buffer.

No new matter is added by the corrections herein. Patentees respectfully request correction of the errors as provided by the attached Certificate of Correction PTO form PTO/SB/44.

The Commissioner for Patents is authorized to charge the fee (\$100) under §1.20(a) to Life Technologies Inc. Deposit Account No. 50-3994. Any deficiency or overpayment should be charged or credited to the deposit account.

Respectfully submitted,

/Gloria L Norberg/

Date: April 22, 2010

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Agent for Patentee

been solved, the corresponding structure of *E. coli* RNase H1 is known (Science. 1990 Sep 21;249(4975):1398-405). However, *E. coli* RNase H1 shares only 30% identity with the MMLV RNase H (Proc Natl Acad Sci U S A. 1986 Oct.; 83(20): 7648-52), which include essential metal binding and active site residues. Several "support" residues not directly involved in catalysis were also identical in the two enzymes. Several mutants of *E. coli* RNase H1 have been identified that exhibit reduced RNase H activity. The data provided herein support the choice of 9-10 mutants that were found to enhance the ability of the RT to maintain template interactions, significantly reduce the RNase H activity of MMLV RT to a level in the 1-50% range as compared to the wild-type MMLV RT, but not having the deleterious effects of deletion mutants or mutants having no RNase H activity. The results disclosed herein demonstrate that reduced activity (but not eliminated or no RNase H activity), is desirable for the aRNA synthesis application and the creation of a hyperactive RT.

[0061] The present inventors have developed a series of point mutants, e.g., H638G MMLV RT, Y586A MMLV RT, D653N MMLV RT, D524N MMLV RT, D524E MMLV RT, and E562D MMLV RT using pSE380 containing the MMLV RT gene (pSE380-MMLV RT) and the mutagenic primers given in Table 1. The nucleic acid sequence for one such mutant is shown in Figure 2, with the amino acid sequence described in Figure 3. Amplification of the mutant sequences was accomplished via PCR using the Quick Change mutagenesis kit (Stratagene). The resulting PCR product was transformed and plated onto solid media containing ampicillin. Plasmid DNA from selected clones was prepared with the QIAprep Spin Miniprep Kit. In the case of Y586A MMLV RT and H638G MMLV RT, the presence of the correct mutation was diagnosed after restriction digest with Sma I. Clones containing D653N, D524N MMLV RT, D524E MMLV RT, and E562D MMLV RT were screened by sequencing. In each case, sequencing across the MMLV gene confirmed the desired mutations.

Table 1. Mutagenic Primers Used to Create H638G MMLV RT, Y586A MMLV RT, and D653N MMLV RT. "F" and "R" refer to "forward" and "reverse" primers, respectively.

H638G-F  
CTTAGCATAATCCATTGTCCCGGGGTCAAAGGGACACAGCGC (SEQ ID NO.: 3);  
H638G-R  
GCGCTGTGTCCCTTTTGACCCCCGGGACAATGGATTATGCTAAG (SEQ ID NO.: 4);  
Y586A-F  
GAAGCTAAATGTTTATACTGATTCCCGGGCTGCTTTTGCTACTGCCC (SEQ ID NO.: 5);  
Y586A-R  
GGGCAGTAGCAAAAGCAGCCCGGGAATCAGTATAAACATTTAGCTTC (SEQ ID NO.: 6);  
D653N-F  
GGCAACCGGATGGCTAACCAAGCGGCCCGAAAG (SEQ ID NO.: 7);

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No. : 10/827,498 Confirmation No. 3464  
Applicant : Chen, *et al.*  
Filed : 04/19/2004  
TC/A.U. : 1652  
Examiner : Richard G Hutson  
Docket No. : 6560 US  
Customer No. : 68163

**M/S RCE**  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

CERTIFICATE OF ELECTRONIC SUBMISSION

DATE: 03/06/2009

**REQUEST FOR CONTINUED EXAMINATION; AMENDMENT;  
RESPONSE TO FINAL OFFICE ACTION MAILED JANUARY 8, 2009**

Sir:

This paper is submitted in response to the Final Office Action mailed January 8, 2009 for which the shortened statutory date for response is April 8, 2009. This paper is being filed prior to the two-month date of March 8, 2009. No request for an extension of time is believed required. However, should such a request be needed, this paper is considered such a request. This response is being filed with a Request for Continued Examination (RCE) under 37 C.F.R. §1.114. The Commissioner is authorized to charge the fee for the RCE (\$405) and any further fees due under 37 C.F.R. §§ 1.16 to 1.21 to Deposit Account No.: 503994/6560US. Applicants respectfully request reconsideration and withdrawal of all outstanding objections and rejections.

**Amendments to the Claims** begin on page 2 of this paper.

**Remarks** begin on page 7.

**I. Listing of the Claims:**

This listing of claims replaces all prior versions or listings of claims in the application:

1. – 128. (Canceled)

129. (Previously presented) An isolated reverse transcriptase protein comprising SEQ ID NO:2.

130. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase may be used in the preparation of full-length cDNA.

131. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase comprises reverse transcriptase produced recombinantly.

132. (Canceled)

133. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase is purified and is greater than 90% pure.

134. (Currently Amended) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 1 ug of an amplified RNA (aRNA) from 100 ng of template RNA in a single amplification reaction.

135. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 5 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.

136. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 7 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.

137. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 10 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.

138. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 15 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.

139. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 25 ug of an aRNA from 100 ng of template RNA in a single amplification reaction.

140. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 1 ug of an aRNA from 10 pg of template RNA after a two-round amplification reaction.

141. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 2 ug of an aRNA from 10 pg of template RNA after a two-round amplification reaction.

142. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 5 ug of an aRNA from 10 pg of template RNA after a two-round amplification reaction.

143. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a yield of greater than about 10 ug of an aRNA from 10 pg of template RNA after a two-round amplification reaction.

144. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a cDNA greater than about 6, 9 or 11 kilobases in a single cDNA synthesis reaction.

145. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a cDNA greater than about 6 to about 15 kilobases in a single cDNA synthesis reaction.

146. (Previously presented) The reverse transcriptase of claim 129, wherein the reverse transcriptase produces a cDNA greater than about 15 kilobases in a single cDNA synthesis reaction.

147. (Previously presented) The reverse transcriptase of claim 129, wherein the DNA polymerase activity is greater than about 200 Units per microgram.

148. (Previously presented) The reverse transcriptase of claim 129, wherein the DNA polymerase activity is between about 0.1 and 300 Units per microgram.

149. (Currently Amended) The reverse transcriptase of claim 129, wherein the RNase H activity is between about 0.1 and about 25 percent of the wild-type MMLV RNase H activity.

150. – 151. (Canceled)

152. (Previously presented) A kit for nucleic acid synthesis, comprising, in a suitable container:

a reverse transcriptase protein of Claim 129; and

a reaction solution for the reverse transcriptase protein.

153. (Previously presented) The kit of claim 152, further comprising an insert that comprises information for using the reverse transcriptase protein.

154. (Currently Amended) The kit of claim 152, wherein the reaction solution comprises a concentrated reverse transcriptase reaction buffer.

155. (Previously presented) The kit of claim 152, further comprising a primer.

156. (Previously presented) The kit of claim 152, wherein the reaction solution comprises a reverse transcriptase buffer.

157. (Previously presented) The kit of claim 152, wherein the reaction solution comprises a PCR buffer.

158. (Previously presented) The kit of claim 152, further comprising a mix of nucleotides.

159. (Previously presented) The kit of claim 152, further comprising containers comprising individual nucleotides.

160. (Previously presented) The kit of claim 152, wherein the reaction solution comprises a buffer for in vitro transcription.

161. (Previously presented) The kit of claim 152, further comprising a template purification column.

162. (Previously presented) The kit of claim 152, further comprising magnetic particles suitable for nucleic acid purification.

163. (Previously presented) A kit for nucleic acid synthesis, comprising, in a suitable container: a reverse transcriptase protein comprising SEQ ID NO:2; and a reaction solution for the reverse transcriptase protein.

164. (Previously presented) A kit for RNA amplification, comprising, in a suitable container: a reverse transcriptase protein comprising SEQ ID NO:2; an oligonucleotide comprising a transcriptional promoter region and oligo(dT) region; a DNA polymerase; and an RNA polymerase.

165. (Previously presented) The kit of claim 164, further comprising an insert that comprises information for using the reverse transcriptase protein.

166. (Previously presented) The kit of claim 164, further comprising a primer.

167. (Previously presented) The kit of claim 164, further comprising a reverse transcriptase buffer.

168. (Previously presented) The kit of claim 164, further comprising a DNA Polymerase buffer.

169. (Previously presented) The kit of claim 164, further comprising a mix of nucleotides.

170. (Previously presented) The kit of claim 164, further comprising containers comprising individual nucleotides.

171. (Previously presented) The kit of claim 164, further comprising a buffer for in vitro transcription.

172. (Previously presented) The kit of claim 164, further comprising a nucleic acid purification column.

173. (Previously presented) The kit of claim 164, further comprising a magnetic particle or particles suitable for nucleic acid purification.

174. (Previously presented) An RT-PCR kit comprising in one or more suitable containers: a reverse transcriptase comprising SEQ ID NO:2, two or more primers, nucleotides, a thermostable DNA polymerase and an RT-PCT buffer.

175. (Currently Amended) The RT-PCR kit of claim 174, wherein the container comprising a reverse transcriptase further comprises one or more further reverse transcriptases in addition to the reverse transcriptase comprising SEQ ID NO:2.

## **II. Remarks**

### **A. Status of the Claims**

Claims 1-128, 132, 150 and 151 are canceled without prejudice to filing in a continuing application. Claims 129-131, 144-148, 152-155, 157-162 and 164-174 are allowed. Claims 134, 149, 154 and 175 have been amended so as to further the present case to allowance. Claims 129-131, 133-149, and 152-175 are pending. Support for amended claim language is provided below. Applicants submit that no new matter has been introduced by the amendments.

### **B. Information Disclosure Statements**

Filed concurrently herewith is a Supplemental Information Disclosure Statement (SIDS) listing patents, publications or other information (listed on the attached modified Form PTO 1449) which may be material to the patentability of this application and in respect of which there may be a duty to disclose in accordance with 37 CFR §1.56. Applicants respectfully request that documents listed in the concurrently-filed SIDS be reviewed by the Examiner and made of record in the application.

### **C. Claim Objections**

The Final Office Action states an objection to Claims 134-143 for the term “aRNA” and to Claims 150 and 151 as improperly dependent upon Claim 129. Final Office Action at page 2.

#### **Response**

Claim 134 has been amended to recite “amplified RNA” to precede “aRNA,” thereby providing the meaning of “aRNA” in the first occurrence thereof as requested by the Examiner and as supported by the specification at paragraph [0020].

Claims 150 and 151 are canceled without prejudice for filing in a continuing application.

Applicants therefore request that the objections to Claims 134-143 and 150-151 be withdrawn.

### **D. Rejection of Claims under 35 U.S.C. §112, Second Paragraph**

#### **Office Action**

The Office Action states a rejection of Claims 132 and 133 as indefinite regarding the terms “purified” and “isolated,” a rejection of Claim 149 as indefinite regarding “wild-type

RNase H activity,” a rejection of Claims 156 and 163 as indefinite for possible duplication of Claim 154, and a rejection of Claims 175 as indefinite for the phrase “one or more further reverse transcriptases.” Office Action at pages 2-4.

**Response**

Applicants traverse this rejection.

Claim 132 is canceled. Applicants submit that Claim 133 is definite due to the recitation of “wherein the reverse transcriptase is purified and is greater than 90% pure” since one of ordinary skill in the art would know how to measure enzyme purity.

Claim 149 has been amended to clarify that the wild-type RNase H activity refers to wild-type MMLV RNase H activity, which language has support in the title of column 3 of Table 4 on page 25 of the specification.

Claim 154 has been amended to recite “the reaction solution comprises a concentrated reverse transcriptase reaction buffer,” which phrase has support at paragraph [0019] of the specification (page 6, line 19) and which phrase distinguishes Claim 154 from Claim 156.

Claim 163 is an independent claim and is not duplicative of Claim 152 in that the reverse transcriptase of Claim 152 is that of Claim 129 which is to an isolated reverse transcriptase protein comprising SEQ ID NO:2.

Claim 175 has been amended to recite “wherein the container comprising a reverse transcriptase further comprises one or more further reverse transcriptases in addition to the reverse transcriptase comprising SEQ ID NO:2,” which language has support in the specification at paragraph [0022], page 7, lines 23-26.

In light of these remarks, Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph, be withdrawn.

**E. Rejection of Claims under 35 U.S.C. §112, First Paragraph**

**Office Action**

The Office Action states a rejection of Claims 150 and 151 for failing to meet the written description and enablement requirements of 35 U.S.C. §112. Office Action at pages 4-9.

**Response**

Applicants traverse this rejection. However, in order to expedite prosecution of the present application to allowance, Applicants have canceled Claims 150-151. Applicants respectfully request that the rejections under 35 U.S.C. §112, first paragraph, be withdrawn.

**F. Rejection of Claims under 35 U.S.C. §102**

**Office Action**

The Office Action states a rejection of Claims 150 and 151 as anticipated by Ruppert (U.S. Patent No. 5,891,637). Office Action at page 9.

**Response**

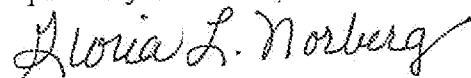
Applicants traverse this rejection. However, in order to expedite prosecution of the present application to allowance, Applicants have canceled Claims 150-151. Applicants respectfully request that the rejection under 35 U.S.C. §102 be withdrawn.

**G. Conclusion**

Applicants believe that the foregoing remarks fully respond to all outstanding matters for this application. Applicants respectfully request reconsideration of the claimed invention and issuance of a Notice of Allowance for Claims 129-131, 133-149, and 152-175.

Should there be any questions or comments regarding this document, the Examiner is invited to contact Applicants' representative, Gloria L. Norberg at 512-721-3654 for discussion.

Respectfully submitted,



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Date: March 6, 2009